

10/539628

JC17 Rec'd PCT/PTO 17 JUN 2005

IN THE CLAIMS

Claim 1 (original): A method for detecting an analyte in a sample comprising the steps of

providing detection probes being labeled with a first reporter, which detection probes are capable of binding to the analyte,

providing a solid support,

providing capture probes being bound or capable of binding to the solid support, which capture probes are capable of binding to the analyte, thus concentrating the analyte on the solid support,

contacting the sample with the detection probes, the solid support and the capture probes, and

detecting the detection probes, wherein

the detection of detection probes is conducted in the presence of quenching probes binding to surplus detection probes not being bound to the analyte and thereby quenching at least partially an emission of the first reporter of said surplus detection probes and/or

the solid support is labeled with a second reporter different from the first reporter, imaging the sample at an emission wavelength of the second reporter, generating a mask obtained from imaging the sample at the emission wavelength of the second reporter and applying this mask to an image of the sample used for detecting the detection probes.

Claim 2 (original): A method for detecting an analyte in a sample comprising the steps of

providing detection oligonucleotides being labeled with a first reporter, which detection oligonucleotides are capable of binding to the analyte,

providing a solid support,

providing capture oligonucleotides being bound or capable of binding to the solid support, which capture oligonucleotides are

capable of binding to the analyte, thus concentrating the analyte on the solid support,

contacting the sample with the detection oligonucleotides, the solid support and the capture oligonucleotides, and

detecting the detection oligonucleotides, wherein

the detection of detection oligonucleotides is conducted in the presence of quenching oligonucleotides hybridizing to surplus detection oligonucleotides not being bound to the analyte and thereby quenching at least partially an emission of the first reporter of said surplus detection oligonucleotides and/or

the solid support is labeled with a second reporter different from the first reporter, imaging the sample at an emission wavelength of the second reporter, generating a mask obtained from imaging the sample at the emission wavelength of the second reporter and applying this mask to an image of the sample used for detecting the detection oligonucleotides.

Claim 3 (currently amended): The method according to claim 1 [or 2] being conducted in a homogeneous format.

Claim 4 (currently amended): The method according to ~~at least one of claim[s] 1 [to 3]~~ wherein the first and/or second reporter is luminescent, in particular fluorescent.

Claim 5 (currently amended): The method according to ~~at least one of claim[s] 1 [to 4]~~ wherein the first and/or second reporter is a dye.

Claim 6 (currently amended): The method according to ~~at least one of claim[s] 1 [to 5]~~ wherein the detection probes, in particular the detection oligonucleotides, are labeled with a first fluorescent dye and/or the solid support is labeled with a second fluorescent dye.

Claim 7 (currently amended): The method according to ~~at least one~~ of claim[s] 2 [to 6] wherein a hybrid between detection oligonucleotides and analyte has a higher melting temperature than a hybrid between detection oligonucleotides and quenching oligonucleotides.

Claim 8 (original): The method according to claim 7 wherein a melting temperature of a hybrid between detection oligonucleotides and analyte is at least 1 °C, more preferably at least 2 °C, even more preferably at least 5 °C and most preferably at least 10 °C higher than a melting temperature of a hybrid between detection oligonucleotides and quenching oligonucleotides under test conditions.

Claim 9 (currently amended): The method according to ~~at least one~~ of claim[s] 2 [to 8] wherein contacting the sample with the detection oligonucleotides is performed under first hybridization conditions allowing the generation of a stable hybrid between detection oligonucleotides and analyte.

Claim 10 (currently amended): The method according to ~~at least one~~ of claim[s] 2 [to 9] wherein contacting the sample with the quenching oligonucleotides is performed under second hybridization conditions allowing the generation of a stable hybrid between surplus detection oligonucleotides not being bound to the analyte and quenching oligonucleotides.

Claim 11 (original): The method according to claim 10 wherein said second hybridization conditions do not destabilize a hybrid between detection oligonucleotides and analyte formed under said first hybridization conditions.

Claim 12 (currently amended): The method according to ~~at least one~~ of claim[s] 1 [to 11] wherein the capture probes, in particular the

capture oligonucleotides, are covalently bound to the solid support.

Claim 13 (currently amended): The method according to ~~at least one of~~ claim[s] 1 [to 11] wherein the capture probes, in particular the capture oligonucleotides, are capable of binding to the solid support via affinity interaction.

Claim 14 (original): The method according to claim 13 wherein the capture probes / capture oligonucleotides comprise a first affinity unit capable of binding to a second affinity unit attached to the solid support.

Claim 15 (original): The method according to claim 14 wherein the first affinity unit is biotin and the second affinity unit is streptavidin or avidin.

Claim 16 (currently amended): The method according to ~~at least one of~~ claim[s] 1 [to 15] wherein the solid support is a bead, a cell, a pollen, or a plurality thereof.

Claim 17 (currently amended): The method according to ~~at least one of~~ claim[s] 1 [to 16] wherein the first reporter labeling the detection probes / detection oligonucleotides differs in its excitation wavelength and/or its emission wavelength from the second reporter labeling the solid support.

Claim 18 (original): The method according to claim 17 wherein the difference in the excitation wavelength and/or emission wavelength between first and second reporter is at least 10 nm, preferably at least 20 nm, even more preferably at least 50 nm and most preferably at least 100 nm.

Claim 19 (currently amended): The method according to ~~at least one~~

~~of~~ claim[s] 2 [to 17] wherein the detection oligonucleotides comprise a linker sequence, linking the sequence of detection oligonucleotide complementary to the analyte with the first reporter.

Claim 20 (currently amended): The method according to ~~at least one of~~ claim[s] 2 [to 19] wherein the capture oligonucleotides comprise a linker sequence, linking the sequence of the capture oligonucleotide complementary to the analyte with the affinity unit or the solid support.

Claim 21 (currently amended): The method according to ~~at least one of~~ claim[s] 1 [to 20] wherein at least two different analytes are being detected by providing at least two different sets of detection probes/detection oligonucleotides and at least two different sets of capture probes/capture oligonucleotides.

Claim 22 (original): The method according to claim 21 wherein the different sets of detection probes/detection oligonucleotides are being labeled with different reporters.

Claim 23 (original): The method according to claim 22 wherein the reporters of one set are identical, have the same excitation wavelength and/or the same emission wavelength.

Claim 24 (currently amended): The method according to ~~at least one of~~ claim[s] 1 [to 23] wherein the image recorded at the emission wavelength of the second reporter is recorded simultaneously with the image used for detecting the detection probes/detection oligonucleotides.

Claim 25 (original): The method according to claim 24 wherein the image recorded at the emission wavelength of the second reporter is corrected such that it spatially matches with the image used for

detecting the detection probes/detection oligonucleotides, or vice versa.

Claim 26 (currently amended): The method according to ~~at least one of claim[s] 1 [to 25]~~ wherein the quenching probes/quenching oligonucleotides have a quenching unit, said quenching unit preferably being a dye.

Claim 27 (currently amended): The method according to ~~at least one of claim[s] 1 [to 26]~~ wherein the first reporter is a donor of a Förster resonance energy transfer (FRET) donor-acceptor-pair and the quenching unit is an acceptor of said donor-acceptor-pair.

Claim 28 (original): The method according to claim 26 wherein the quenching unit is a dark quencher which quenches at least partially the emission of the first reporter by dissipating an energy of an excited state of the first reporter into the environment.

Claim 29 (currently amended): The method according to ~~at least one of claim[s] 1 [to 28]~~ comprising the step of quantifying the analyte.

Claim 30 (original): The method according to claim 29 wherein the quantification is performed by determining an amount of detection probes/detection oligonucleotides bound to the analyte.

Claim 31 (currently amended): The method according to claim 30 wherein the amount of detection probes/detection oligonucleotides bound to the analyte is expressed as the emission intensity emitted by the first reporter.

Claim 32 (currently amended): The method according to claim 30 [or 31] comprising the step of determining an intensity of a background emission in the vicinity of the solid support and considering such

intensity when determining the amount of detection probes/detection oligonucleotides.

Claim 33 (currently amended): The method according to ~~at least one of claim[s] 1 [to 32]~~ wherein the image of the sample used for detecting the detection probes/detection oligonucleotides is acquired at the emission wavelength of the first reporter.

Claim 34 (currently amended): The method according to ~~any one of the preceding claims~~ claim 1 wherein the detection probes are aptameres, oligonucleotides, or antibodies.

Claim 35 (currently amended): The method according to ~~any one of the preceding claims~~ claim 1 wherein the analyte is a protein or a nucleic acid.

Claim 36 (currently amended): The method according to ~~any one of the preceding claims~~ claim 1 wherein the sample is a cell lysate, in particular a crude cell lysate, or an in vitro prepared sample.

Claim 37 (original): The method according to claim 21 wherein the capture probes/capture oligonucleotides of different sets are attached or capable of binding to different solid supports.

Claim 38 (original): The method according to claim 37 wherein the solid supports differ in the affinity units attached thereto, which affinity units interact with affinity units of the capture probes/capture oligonucleotides.

Claim 39 (currently amended): The method according to ~~any of the preceding claims~~ claim 1 comprising adding a potentially pharmaceutically active substance or a known drug to a cellular sample and analyzing whether such substance induces, inhibits or otherwise modulates the generation of the analyte.

Claim 40 (currently amended): Use of the method according to any of
the preceding claims claim 1 in screening for potentially
pharmaceutically active substances, in diagnostics, or in
determining any potential side effects of drugs.